## CULEX MALARIAGER, N. SP. (DIPTERA: CULICIDAE) FROM DOMINICAN AMBER: THE FIRST FOSSIL MOSQUITO VECTOR OF PLASMODIUM

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Abstract.—A **new species** of fossil mosquito, *Culex malariager* (Culicidae: Diptera), is described from Dominican amber. This species differs from extant members of the genus by the following combination of characters: 1) proboscis shorter than antennae; 2) wing scales both long and narrow and short and broad; 3) base of R vein (remigium) with two groups of dorsal setae; 4) clypeus as long as broad; 5) empodium on foretarsus; 6) vein 2A with rows of erect, short setae, and 7) postgenital lobe covering base of cerci. Inside the body cavity of *C. malariensis* are various stages (oocysts, sporozoites, ookinete, and microgametocyte) of a *Plasmodium* malarial parasite. This is the first record of a fossil mosquito vector of a vertebrate pathogen.

Key Words: Culex malariager n. sp., Culicidae, Dominican amber, malaria vector, Plasmodium

During an ongoing investigation of fossil haematophagous insects, a species of *Culex* in Dominican amber was discovered to contain sporogonic stages of a *Plasmodium* within its body cavity. The description of the *Plasmodium* will be reported in a separate paper (Poinar, in press). The present study describes this fossil mosquito as a new species in the genus *Culex* L.

Although not abundant, fossil culicids are known from Tertiary amber (Poinar 1992) and a single specimen has been described from Cretaceous amber, along with a critical synopsis of previously described fossil mosquitoes (Poinar et al. 2000). Whereas *Culex* is the most common genus of mosquitoes in Dominican amber (Poinar and Poinar 1999), the only described mosquito from these deposits is *Anopheles dominicanus* Zavortink and Poinar, 2000. Dating of Dominican amber is still controversial with the latest proposed age of 20–15 mya based on foraminifera (Iturralde-

Vincent and MacPhee 1996) and the earliest as 45–30 mya based on coccoliths (Cêpek in Schlee 1999). What makes dating of the amber difficult is that it is secondarily deposited in turbiditic sandstones of the Upper Eocene to Lower Miocene Mamey Group (Draper et al. 1994). The plant species that formed the amber is a member of the legume family (*Hymenaea protera* Poinar 1991) and the original environment was similar to a present day moist tropical forest (Poinar and Poinar 1999).

### MATERIALS AND METHODS

The piece of amber originated from mines in the northern area of the Dominican Republic, between the cities of Puerto Plata and Santiago. It is trapezoidal in outline, measuring  $14 \times 17 \times 25 \times 15$  mm on the sides, 5 mm in thickness and weighs 2 grams. Observations and photographs were made with a Nikon stereoscopic microscope SMZ-10 R and Nikon Optiphot<sup>®</sup> at mag-

nifications up to 600×. All measurements are in microns unless otherwise noted.

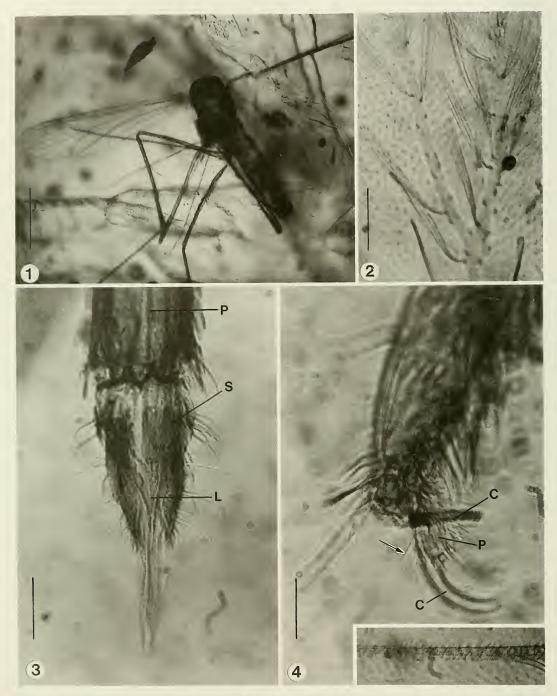
The fossil female mosquito is essentially complete, but somewhat distorted with many scales and bristles detached (except for those on the wings). Only three legs are attached to the body (both forelegs and one midleg), however the others are adjacent to the specimen. The thorax and abdomen are partially cleared, thus making it possible to see internal structures. Since the fossil could not be identified with any extant species it is described as a new species below.

# Culex malariager Poinar, new species (Figs. 1–10)

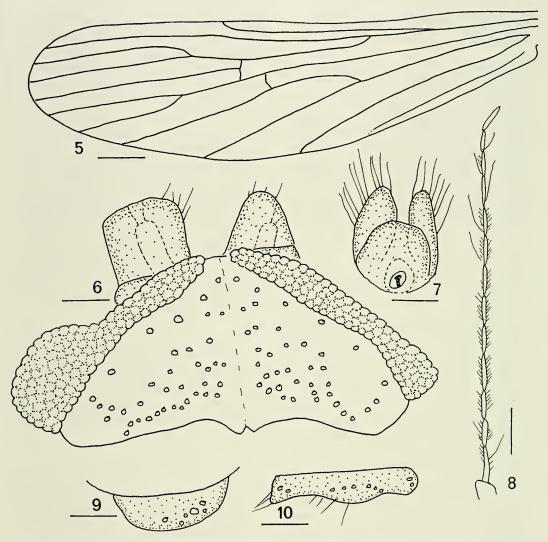
Description.—Size small, head bent down partly under thorax; length from pronotum to tip of abdomen 2.9 mm (however head, thorax and abdomen measured separately, length 4.1 mm).

Head: Length 0.59 mm, width 0.66 mm; vertex and occiput bearing 76 large aveoli (38 on each side; positions vary slightly from side to side); clypeus rounded in front, without scales or setae, length 136 µm, width 133 µm; eyes nearly contiguous with narrow interocular space; eye facets measuring approximately 18 x 21 µm; maxillary palpus with 3 palpomeres, no banding obvious, palpomeres 2 and 3 bear setae and few small scales, palpomere 1 and palpifer bare; lengths, palpomere I (with palpifer) 95 μm; palpomere 2, 123 μm; palpomere 3, 155 µm; proboscis length 1.53 mm, proboscis covered with mostly broad, but some narrow scales; labellum length 136 μm, labellum width 35 µm; distal and proximal sclerites of labellum apparently fused; ligula pointed; antenna length 1.90 mm; scape bare, length 32 µm; pedicel globose, with few setae; pedicel length 116 μm, pedicel width 123 µm; flagellomeres verticillate, however most setae absent; basal and terminal flagellomeres equal in length; lengths of flagellomeres: 1, 175 μm; 2, 140 μm; 3, 168 μm; 4, 161 μm; 5, 161 μm; 6, 154 μm; 7, 147µm; 8, 154 µm; 9, 154 µm; 10, 140 μm; 11, 98 μm; 12, 105 μm; 13, 175 μm.

Thorax: Length 1.22 mm; antepronotum narrow, with 6 aveoli; scutal fossa (prescutum) with 6 aveoli; scutum with both setae and alveoli; scutellum (length 85 µm; width 372 µm) with 3 lobes bearing 10 aveoli; no spiracular, post- spiracular, acrostichal or postnotal setae; 3 prealar setae and 4 aveoli on mesokatepisternum; 2 lower mesepimeral setae; wing length 2.7 mm, greatest width 0.7 mm; microtrichia present, posterior margin of wing with fringe of scales; wing banding not visible; cell R, longer than its stem (R 2+3); cell M almost twice as long as its stem; br and bm cells extend to middle of wing; anal vein terminates well past fork of CuA; plume scales on veins R<sub>2</sub> and R<sub>3</sub> narrow to slightly widened; Sc and R contiguous for 360 µm from wing base; base of vein R with 2 groups of dorsal setae; group 1 with 7 setae 32-79 μm long occurring 204 μm from wing base, group 2 with 8 setae 23-57 µm long occurring 45 µm from wing base; veins Rs and R 4+5 without basal spurs; crossveins without scales; r-m distad to m-cu; alula (length 196 µm) with outer row of scales; no setae on lower surface of wing at base of subcostal vein; scales on wing veins vary in length and breadth, scales on vein R, R  $_{2+3}$ ,  $R_2$  and  $R_3$ , distal portions of veins  $A_1$ and CuA<sub>1</sub> long and narrow, scales on R<sub>1</sub>, R  $_{4+5}$ ,  $M_1$ ,  $M_2$  and basal portions of vein  $A_1$ and CuA, short and broad; other veins with intermediate scales; vein 2A without scales but bearing rows of short setae 7-10 µm in length (setae differ in structure and size from wing microtrichia); halter length 315 um; legs brown-scaled, no pattern or ornamentation visible; forecoxa with few alveoli near apex; forefemur swollen slightly at base, length 1.38 mm; foretibia 1.8 mm; ventral surface of foretarsomere 1 with short spine, opposite surface with scales and longer spines; length of tarsomeres of right fore tarsus; 1, 1.5 mm; 2, 0.57 mm; 3, 0.36 mm; 4, 0.157 mm; 5, 0.14 mm; claw length 50 µm; pulvillus length 20 µm; foretarsus with slender empodium arising between pulvilli, its filament short with a sin-



Figs. 1–4. *Culex malariager* in Dominican amber. 1, Anterolateral view. Bar = 0.9 mm. 2, Scales at the base of veins R2 and R3. Bar = 45  $\mu$ m. 3, Labella with prementum (P), closely adjacent distal and proximal sclerites (S) and ligula (L). Bar = 45  $\mu$ m. 4, Foretarsomere with simple claw (C), pulvillus (P) and empodium (arrow). Bar = 23  $\mu$ m. Insert shows a portion of vein 2A bearing rows of short, erect setae. Bar = 30  $\mu$ m.



Figs. 5–10. *Culex malariager* in Dominican amber. 5, Right wing (ventral view) with venation. Bar = 210  $\mu$ m. 6, Dorsal surface of head with aveoli on occiput and vertex. Bar = 70  $\mu$ m. 7, Cerci, postgenital lobe, and gonotreme. Bar = 49  $\mu$ m. 8, Right flagellomeres. Bar = 252  $\mu$ m. 9, Aveoli on antepronotum. Bar = 70  $\mu$ m. 10, Aveoli on trilobed scutellum. Bar = 114  $\mu$ m.

gle branch, basal sclerite not visible, empodium not seen on mid- and hindtarsi, all pulvilli with fine setae on lower surface; midtarsomere 5 length 0.13 mm; claw length 46  $\mu$ m; pulvillus length 17  $\mu$ m; hindtarsomere 5 length 0.15 mm; claw length 23  $\mu$ m; pulvillus length 18  $\mu$ m; all claws paired, simple, equal.

Abdomen: Length 2.3 mm; covered with mostly short, broad scales; tergum 8 with

very numerous long setae and scales surrounding cerci; cercus length 50  $\mu$ m; cercus width at base 26  $\mu$ m; postgenital lobe large, covering bases of cerci.

Ratios: Palpus to proboscis 0.24; proboscis to antenna 0.81; palpus to clypeus 2.7; proboscis to forefemur 1.11; clypeus to pedicel 1.17.

Material examined.—Holotype female in Dominican amber, deposited in the Poinar

amber collection maintained at Oregon State University (accession number D-7–6B).

Type locality.—La Toca amber mine in the Dominican Republic.

Etymology.—"malariager" is based on the vector association of the fossil mosquito with *Plasmodium*. It is formed with the Latin suffix –ger meaning bear, carry or have.

Diagnosis.—The following characters place C. malariager in the genus Culex: 1) short palps; 2) tip of abdomen blunt; 3) scutellum trilobed with each lobe bearing separate tufts of setae; 4) no spiracular or postspiracular setae; 5) short, partly concealed cerci; 6) claws simple; 7) pulvilli present; 8) vein 1A long; 9) wing microtrichia distinct; 10) eyes nearly contiguous; 11) terga and sterna II-VII uniformly scaled; 12) cell R2 longer than vein  $R_{2+3}$ ; and 13) hairs on underside of costal vein absent. It is not possible to assign this species to an extant subgenus since these taxa are defined on the types of scales found at particular locations, scale and setal positions on the thorax, and color of scales, abdomen and pleural integument (Berlin and Belkin 1980, Casal and Garcia 1968, Harbach and Peyton 1992), most of which cannot be determined in the fossil.

The species can be distinguished from extant *Culex* by the following combination of characters: 1) proboscis shorter than antennae; 2) wing scales both long and narrow and short and broad; 3) base of vein R (remigium) with two groups of dorsal setae; 4) clypeus as long as broad; 5) empodium on foretarsus; 6) vein 2A with rows of erect, short setae; and 7) postgenital lobe covering base of cerci. Especially significant are items 3 and 6 since they do not appear to have been reported previously on any *Culex* spp. (Tom Zavortink, personal communication).

#### DISCUSSION

Two other *Culex* fossils are known from North America, *C. damnatorum* Scudder, 1890 from Eocene Green River deposits in Wyoming and *C. winchesteri* Cockerell, 1919 from Eocene deposits in Colorado. Neither of these is similar to *C. malariager*.

There were two other hematophagous invertebrates in the same piece of amber, a triatomid bug and a female Ornithodorus dominicana Poinar, 1995. The presence of these three fossils suggests that the resin was produced in or close to a domicile of vertebrates, which probably served as hosts to these parasites. Since the stages of the malarial parasite inside C. malariager resemble those of an extant avian Plasmodium species (Poinar, in press) and it is known that Culex transmit avian malaria (Riper et al. 1994), at least one of the vertebrate hosts of C. malariager was a bird. This is the first record of a fossil mosquito vector of a vertebrate pathogen.

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